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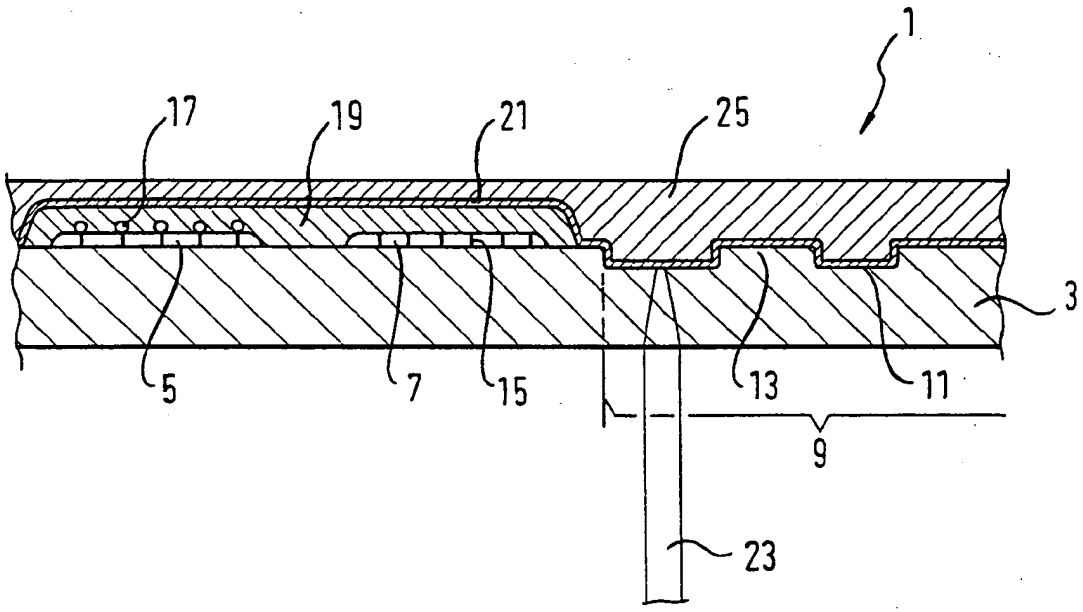
(54) Titre : PROCEDE DE DETECTION D'ANALYTES DANS UN ECHANTILLON POUR ESSAI ET SUPPORT DE MESURE Y RELATIF

(54) Title: METHOD OF DETECTING ANALYTES IN A SAMPLE AND SUPPORT FOR THIS PURPOSE




(57) Abrégé/Abstract:
The invention relates to a method of detecting analytes in a sample. According to said method, analyte-specific binders (15) are immobilized on a plurality of detection fields (5, 7) on one of the planar faces of a disk-shaped substrate (3). The sample is contacted with said detection fields (5, 7) and the presence or/and the quantity of the analytes (17) to be detected is determined by optically evaluating the detection fields (5, 7). According to the invention, a substrate (3) is used that is produced from an optically transparent material. The detection fields (5, 7) are arranged along at least one spiral line (27) distributed across the substrate (3). After the sample has been contacted with the detection fields (5, 7), a reflecting layer (21) is flatly applied on the substrate (3) on the planar face carrying the detection fields (5, 7). Alternatively, a magnetic read-out of the substrate can be made instead of the optical read-out.

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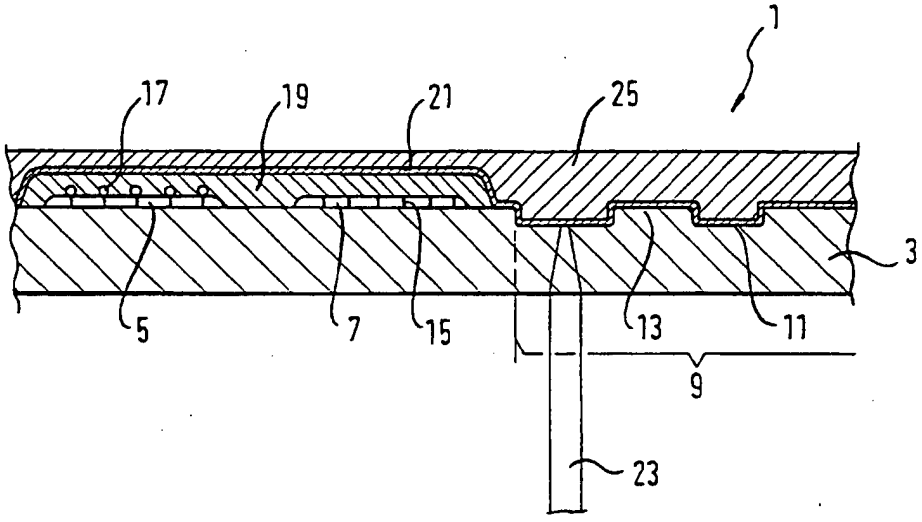
*[Fortsetzung auf der nächsten Seite]*

(54) Title: METHOD OF DETECTING ANALYTES IN A SAMPLE AND SUPPORT FOR THIS PURPOSE

(54) Bezeichnung: VERFAHREN ZUM NACHWEIS VON ANALYTEN IN EINER MESSPROBE SOWIE MESSTRÄGER HIERFÜR




(57) Abstract: The invention relates to a method of detecting analytes in a sample. According to said method, analyte-specific binders (15) are immobilized on a plurality of detection fields (5, 7) on one of the planar faces of a disk-shaped substrate (3). The sample is contacted with said detection fields (5, 7) and the presence or/and the quantity of the analytes (17) to be detected is determined by optically evaluating the detection fields (5, 7). According to the invention, a substrate (3) is used that is produced from an optically transparent material. The detection fields (5, 7) are arranged along at least one spiral line (27) distributed across the substrate (3). After the sample has been contacted with the detection fields (5, 7), a reflecting layer (21) is flatly applied on the substrate (3) on the planar face carrying the detection fields (5, 7). Alternatively, a magnetic read-out of the substrate can be made instead of the optical read-out.

*[Fortsetzung auf der nächsten Seite]*

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*Zur Erklärung der Zweibuchstaben-Codes, und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.*

**(57) Zusammenfassung:** Es wird eine Verfahren zum Nachweis von Analyten in einer Messprobe vorgeschlagen, bei dem auf einem scheibenförmigen Substrat (3) auf einer seiner Flachseiten analytspezifische Binder (15) in einer Vielzahl von Nachweisfeldern (5, 7) immobilisiert werden, sodann die Messprobe in Kontakt mit den Nachweisfeldern (5, 7) gebracht wird und anschließend das Vorhandensein oder/und die Menge der nachzuweisenden Analyten (17) durch optische Auswertung der Nachweisfelder (5, 7) ermittelt wird. Erfindungsgemäss wird ein aus einem optisch transparenten Material gefertigtes Substrat (3) verwendet, wobei die Nachweisfelder (5, 7) längs mindestens einer Spirallinie (27) verteilt auf dem Substrat (3) angeordnet werden. Nach Inkontaktbringung der Messprobe mit den Nachweisfeldern (5, 7) wird das Substrat (3) auf seiner die Nachweisfelder (5, 7) tragenden Flachseite flächig mit einer Reflexionsschicht (21) versehen. Alternativ ist statt der optischen Auslesung auch eine magnetische Auslesung des Substrats denkbar.

New Claims

1. Method for the detection of analytes in a sample, where analyte-specific binders (15) are immobilized in a plurality of detection fields (5, 7) located on one of the planar faces of a disk-shaped substrate (3), then the samples are contacted with the detection fields (5, 7), and subsequently the presence and/or the quantity of the analytes (17) to be detected is (are) determined by optical evaluation of the detection fields (5,7), where a substrate (3) prepared from an optically transparent material is used and where the detection fields (5, 7) are arranged along at least one spiral line to (27) and/or a plurality of concentric circular lines on the substrate (3), characterized in that, after contacting the sample with the detection fields (5, 7), an optical reflecting layer (21) is applied over the detection fields (5, 7) on the planar face of the substrate (3) which carries the detection fields (5, 7).

2. Method according to Claim 1,
characterized in that the reflecting layer (21) is made of aluminum.

3. Method according to one of Claims 1 and 2,
characterized in that, with reference to the disc axis, radially adjacent detection fields (5, 7) are arranged with radial separation.

4. Method according to one of Claims 1-3,
characterized in that, along the spiral line (27) or a circular line, adjacent detection fields (5, 7) are arranged with separation from each other.

5. Method according to one of Claims 1-4,
characterized in that, on the planar face of the substrate (3) which carries the detection fields (5, 7), along the spiral line (27), or at least along one circular line, additional data fields (9) are formed, which represent information pertaining to samples and/or detection fields and/or the evaluation.

6. Method according to Claim 5,
characterized in that detection fields (5, 7) and data field (9) are arranged alternately along the spiral line (27) or along at least one circular line.

7. Method according to Claim 5,
characterized in that detection fields and data fields are each formed on separate circular lines.

8. Method according to one of Claims 5-7,
characterized in that for the formation of the data fields (9), recesses (11) are formed in the planar face of the substrate (3) which carries the detection fields (5, 7) and in that the reflecting layer (21) is applied in such a manner that it reaches into the recesses (11).

9. Method according to one of Claims 5-7,
characterized in that for the formation of the data fields, a substance which influences incident reading light is applied on the planar face of the substrate which carries the detection fields.

10. Method according to one of Claims 1-9,
characterized in that, on the planar face of the substrate (3) which carries the detection fields (5, 7), at least one reference field, whose optical properties are used as reference in the evaluation of the detection fields (5, 7) along the spiral line (27) or along at least one circular line is formed in addition.

11. Method according to one of Claims 1-10,
characterized in that, after contacting the sample with the detection fields (5, 7), before the application of the reflecting layer (21), a coating layer (19) made of an optically transparent material is applied on the detection fields (5, 7).

12. Method according to Claim 11,
characterized in that for the coating layer (19) a polymer-based material is used.

13. Method according to one of Claims 1-12,
characterized in that a substrate (3) made of polycarbonate is used.

14. Method according to one of Claims 1-13,
characterized in that the substrate (3) is provided at a manufacturing site with the binders (15), dried and packaged, and in that the substrate (3) so prepared is then brought to an application site which is at a distance from the manufacturing site, at which application site the sample is contacted by a user with the detection fields (5, 7).

15. Method according to one of the preceding claims,
characterized in that the detection of the analytes is carried out by a detection of a change in the optical properties of the detection fields.

16. Method according to Claim 15,
characterized in that the optical change in the detection fields is caused by isotopes, enzymes, fluorochromes, dyes, metal colloids and/or beads.

17. Method according to Claim 16,
characterized in that latex beads, plastic beads, glass beads and/or metal beads are used.

18. Support for use with the method according to one of Claims 1-17,
comprising a disc-shaped substrate (3) made of an optically transparent material, to one of whose planar sides analyte-specific binders (15) are immobilized in a plurality of detection fields (5, 7), where the detection fields (5, 7) are arranged along at least one spiral line (27) and/or a plurality of concentric circular lines on the substrate (3), characterized by a reflecting layer (21) being flatly applied over the detection fields (5, 7), on the planar face of the substrate which carries the detection fields, after contacting the sample with the detection fields (5, 7).

19. Support according to Claim 18,
characterized in that a protective layer (25) is flatly applied on the reflecting layer (21).

20. Support according to Claim 19,

characterized in that the protective layer (25) is made of an acrylate-based material.

21. Support according to one of Claims 18-20,
characterized in that it is made available as a packaged commercial unit, prior to the application of the reflecting layer.

22. Support according to Claim 21,
characterized in that it is packaged in the dry state.

23. Method for the detection of analytes in a sample, in which analyte-specific binders are immobilized in a plurality of detection fields on at least one of the planar faces of a disc-shaped substrate, then the sample is contacted with the measurement fields, and subsequently the presence and/or the quantity of the analytes to be detected is (are) determined by evaluation of the detection fields, where the detection fields are magnetically evaluated and, for that purpose, binders, or the analytes to be detected, are labelled with magnetic and/or magnetizable labels and the detection fields are arranged along a plurality of concentric circular lines and/or along at least one spiral line on the substrate, characterized in that, prior to the formation of the detection fields or after contacting the sample with the detection fields, a magnetic layer containing magnetic and/or magnetizable particles is flatly applied over each planar face of the substrate which carries detection fields.

24. Method according to Claim 23,
characterized in that, after contacting the sample with the detection fields, a fixation layer is applied to the detection fields.

25. Method according to Claim 24,
characterized in that the fixation layer is flatly applied on each planar face of the substrate which carries detection fields.

26. Method according to Claim 24 or 25,
characterized in that, for the fixation layer, a polymer-based material is used.

27. Use of a support according to one of Claims 18-22 in an immunoassay and/or nucleic acid hybridization assay and/or lectin-sugar assay and/or protein-nucleic acid assay.

Method of detecting analytes in a sample and support for this purpose

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AMENDED PAGE

New Description

The invention relates to the detection of analytes in a sample. Examples of possible analytes are proteins, peptides, nucleic acids and their derivatives, as well as molecules which can bind to them.

A potential sample is any sample which is suspected to contain at least a part of the sought analytes. Examples include a blood sample, a serum sample and/or a urine sample, or in general any solution which contains the sought analyte.

For the detection of analytes in a sample, it is known to use so-called binding assays. In such binding assays, in general, analytes are detected by their specific interactions with receptors. Examples of such interactions are protein/protein interactions which occur, for example, during gene expression, enzyme/substrate or enzyme/effector interactions which occur in metabolism, or protein/DNA or protein/RNA interactions which occur during gene expression. A systematic understanding of these molecular interactions is a prerequisite for the understanding of all biological processes both in normal and in diseased cells.

Furthermore, binding assays can be used to detect nucleic acids. DNA/DNA interactions play a principal role in molecular biology, particularly in the identification of genes and in strategies for mapping DNA, in the detection and quantification of gene expression as well as in the molecular diagnosis or therapy of diseases. Naturally, DNA/RNA and RNA/RNA interactions are also possibilities.

Because of the large number of genes, proteins, chemical effectors and RNA aptamers, an understanding of or intervention in biochemical processes often requires the identification, quantification or classification of a plurality of molecular interactions or the identification of effective interaction partners from a large number of possible combinations. The screening and analyzing of a large number of molecular interactions is, however, frequently limited with the methods known in the state of the art.

Often, samples are to be examined for a plurality of analytes. Consequently, the problem of large data quantities for evaluation arises. However, to be successful in practice, the evaluation must be possible in a reasonable time period. Known analysis systems have been shown to be only satisfactory to a limited degree.

From an article entitled "Metal Nano Clusters as Transducers for Bioaffinity Interactions" by Thomas Schalkhammer in Monatshefte für Chemie 000, 1-26, Springer-Verlag 1998, pp. 1-

26, a sensor setup is known for biorecognitive binding processes and catalytic-enzymatic processes, in which a mirror layer made of silver is arranged under a polycarbonate substrate, and on top of it a separation layer made of a polymer material is arranged. The separation layer serves as the carrier for the binders which are immobilized on it. The evaluation is carried out with the aid of metal clusters, by means of which the analytes to be detected are marked, and then are engaged in an electromagnetic interaction with the metallic mirror layer. In sensor areas with strong overlap with bound metal clusters, light, which is radiated from the side of the mirror layer which is turned away from the polycarbonate substrate, is absorbed more strongly than in overlap-free or less overlapping sensor areas, which makes it possible to make an optical read-out of the sensor via absorption measurements.

For the functioning of these known sensors, the thickness of the separation layer is a decisive parameter. This thickness must be chosen in a predetermined range, which is dependent on the wavelength of the light irradiated during the subsequent evaluation. In particular, one must ensure that the separation layer has an even thickness throughout its entire extent. To achieve this requirement, high manufacturing technology costs are required.

In the article referred to, reading devices, including CD-ROM driver, that make it possible to manage even relatively large amounts of data are already proposed for the optical evaluation of this sensor.

From WO96/09548, a circular disk-shaped support is known, on whose surface an analyte-specific binder is immobilized. A reflecting layer is incorporated in the support; otherwise, the support consists of an optically transparent material. For the detection of analytes which adhere to the binders, an optical reading device is used, which scans, in a spiral pattern, the surface of the support, which is provided with the binders, using a laser beam.

From WO98/12559 a circular disk shaped support is also known, to whose surface analyte-specific binders are immobilized in a plurality of detection fields, which are distributed along circular or spiral tracks. The detection fields are arranged on a substrate layer of the support, on whose opposite side a reflecting layer is located. To read the detection fields, a laser beam is again used, which scans, as does a CD-ROM, the side of the support which carries the detection fields.

Moreover, from WO98/01533, it is known to mark the binder molecules located in detection fields on a circular disk-shaped support with magnetic markers, and to evaluate the detection fields by means of a magnetic reading procedure.

The problem of the invention is to indicate a way which allows the examination of a sample for a large number of analytes at lower cost, not only with regard to the data technology used for evaluation, but also with regard to the providing of the sensor.

For the solution of this problem, a method according to the invention is provided according to Claim 1.

In the invention, the detection fields can be formed directly on the substrate. Here it is recommended to pretreat the substrate surface first chemically, or to effect a modification of the surface by a pretreatment with an oxygen plasma. This modification can facilitate the application of the binder for the formation of the analyte-specific detection fields. Accordingly, no separation layer with a thickness which must be precisely maintained needs to be applied on the substrate under the binder according to the invention, as was necessary in the sensor known from Schalkhammer. Consequently, the cost of the manufacturing technology is reduced.

The reflecting layer is required for the optical evaluation of the assay. It is applied, after the completion of the biomolecular process steps, to the substrate over the detection fields. Because of their good reflection capacities, it is recommended to use metallic materials. In principle it is also conceivable to use other materials, for example, dielectrics. It is preferred to form the reflecting layer from aluminum. It is also conceivable to use silver as the material for the reflecting layer. The reflecting layer can be formed by chemical vapor phase application. However, it is also possible to apply by gluing a prefabricated reflective film on the substrate.

The optical transparence of the substrate, together with the reflecting layer which is arranged on the side of the detection fields away from the substrate, allows the use of the hardware of commercial CD reading devices for the optical evaluation of this biomolecular sensor according to the invention. In particular in the case of a spiral arrangement of the detection fields, such commercial CD reading devices can be modified at relatively low cost, to allow the desired information to be filtered out of the reflected light of the laser beam which scans the detection fields. It is conceivable that this process requires only software modifications. Even in the case of a circular arrangement of the detection fields, the technology of such CD reading devices can in principle be used, provided the construction and software prerequisites have been created to allow the laser beam which scans the detection fields to jump from circular line to circular line.

It is particularly appropriate to use a CD-ROM reader, which, if embedded in a computer architecture, allows the user the possibility to define the evaluation software himself/herself.

The evaluation of the detection fields can yield quantitative as well as qualitative information, depending on the chosen evaluation algorithm. Using appropriate labels, the optical properties, in particular the absorption behavior, of detection fields in which the analytes have become bound to receptors, can be made distinguishable from detection fields to whose receptors no analytes have become bound. In this manner it is possible, for example, via absorption measurements, to recognize those detection fields which carry analytes after the completion of the biomolecular process steps. First, this would constitute purely qualitative information. If, in

addition, one wishes to obtain quantitative information, it is conceivable to scan the detection fields several times and to change, stepwise from scanned passage to scanned passage, a threshold which is taken during the evaluation as the decision limit between the presence and absence of analytes. In this manner, statements can also be made regarding the quantity of the analytes which remain bound in the different detection fields.

If the assay read out uses an optional suitably adapted CD-ROM drive, a connected computer allows the user to further process the data quantity produced by the drive, where the user can store the results of this further processing in external data storage devices, for example, to prepare data banks on patients. For the data storage, conventional standard storage devices can be used, for example, hard discs or floppy discs. With the aid of the computer, it is possible to carry out a rapid evaluation of the data stream of the drive. The capacity of conventional computers and storage systems is generally sufficient, even for complex gene analyses.

As a rule it will be desirable to obtain sharply delimited detection fields, which, in addition, should be very small, to allow the accommodation of a sufficient number of detection fields on the substrate disc, which is advantageously the size of a conventional CD (compact disc). If one starts with the assumption that the radial separation between two successive spiral windings is advantageously approximately 1.6 μm, then it is recommended to apply the binders in fields which have a radial width of not more than 1 μm, so that a sufficient separation exists between radially adjacent detection fields, with reference to the axis of the disc, and each individual detection field as such can be resolved as to location. It is even preferred that the detection fields are radially smaller than 1 μm, where it can be advantageous to form them so they have a radial width which corresponds essentially to that of the width of the data depressions burned into conventional CDs (so-called pits), namely approximately 0.5 μm. At the same time it is recommended to arrange detection fields which are adjacent to each other at intervals from each other along the spiral line or along a circular line, to allow the individual resolution by the evaluation system. The detection fields can be in the form of essentially square or circular surfaces. It is also conceivable to form them longitudinally, in accordance with the shape of the data pits of conventional CDs in the circumferential direction, that is in the spiral or circular line direction, with an approximately oval or rectangular shape.

To apply the binders under the above outlined dimensional framing conditions for the detection fields on the substrate, it is particularly suitable to use microprinting techniques, ink jet techniques or electrospray techniques. For further information, with regard to the microprint techniques, reference is made to: "Microfabrication, Microstructures and Microsysterns" by Dong Qin et al., "Topics in Current Chemistry," Volume 194, 1998, pp. 6 ff., Springer-Verlag. With regard to the ink jet techniques, reference is made, for example, to: "A new device for multifunctional dosage of liquids by a free jet" by N. Hey et al., Proceedings IEEE-Mems, 1998,

CH 36176. Reference studies concerning electrospray techniques, particularly nanoelectric spray techniques, are, for example: "Analytical Properties of the Nanoelectrospray Ion Sources" by M. Wilm and M. Mann in Analytical Chemistry, Volume 68, No. 1, January 1, 1996, pp. 1-8, as well as "Electrospray and Taylor-Cone theory, Dole's beam of macromolecules at last?" by M. Wilm, M. Mann in International Journal of Mass Spectrometry and Ion Processes 136 (1994), pp. 167-180. These techniques make it possible to provide the detection fields with predetermined binders in a targeted manner with high resolution of location. The binders can be applied to the external surface of the substrate disc. It is also conceivable, by analogy to the data pits of conventional CDs, to provide small recesses in the external surface of the substrate, and to immobilize the analyte-specific binders therein. If such recesses for the binders are provided, it will be advantageous to form them so they have a radial width of approximately 0.5 μm, corresponding to the width of the data pits of conventional CDs.

To examine biological interactions at the molecular level, the analytes that are considered, in particular, are nucleic acids and/or proteins. Thus, the method according to the invention can be used to detect protein/protein interactions, such as, for example, those which occur in gene expression or as cell signals, enzyme/substrate or enzyme/effect interactions during the metabolism, or protein/DNA or protein/RNA interactions during gene expression.

By means of an appropriate choice of the binders in the detection fields, DNA/DNA interactions can also be detected, which is of particular significance in gene identification and in the mapping of genes, in the examinations and quantification of gene expression, as well as in molecular diagnosis and/or therapy of diseases.

Molecular interactions and enzyme activities can be influenced by the formation of RNA aptamers and small chemical bonds to macromolecules. Natural and synthetic effector molecules therefore frequently allow the manipulation of biochemical processes by modulating, or intervening in, macromolecular interactions.

The substrate material preferably is a nonporous material. The use of a nonporous carrier allows the defined application of even small detection fields, so that a miniaturization of the test format or the application of a plurality of detection fields is possible. Suitable materials for the disc-shaped substrate are, for example, plastics and glass. For CD-ROM drive applications the preferred material is a focusing material, particularly a polycarbonate.

The immobilization of the binders to the detection fields can be carried out by conventional methods. The immobilization strategy depends on the type of molecule to be immobilized and the given substrate. In general, binders can be bound directly to a matrix by a chemical reaction, for example, via a specific amino acid in a protein (particularly cysteine lysine) or via the phosphate backbone of a DNA molecule. The immobilization can also be carried out with bifunctional chemical crosslinking agents or via a specific interaction with high

affinity, such as the biotin/streptavidin interaction. The analyte-specific binders can also be adsorbed to the surface, where, however, a covalent bond is preferred. As binders, substances and/or molecules are applied to the surface of the substrate which are capable of binding the desired analyte specifically, and, in particular, with high affinity.

After contacting the sample with the detection fields, the presence and/or quantity of the analytes to be detected is determined by optical evaluation of the detection fields. In this context, all the methods known to a person skilled in the art can be used. A conventional method for the analysis of molecular interactions consists of several steps: a first interaction partner, for example, an analyte-specific binder or receptor is linked covalently or adsorptively to a solid support. At the time of the contacting of the sample with the detection fields, a second interaction partner, for example, the analyte, can interact with the receptor. Then, the presence of the analyte (or the absence in competitive test formats) at the location where the receptor is immobilized is detected. The interaction between the two binders is preferably carried out under conditions where both reactants are present in a native, active configuration, preferably in a liquid reaction. It is particularly preferred to carry out the contacting at a pH which is close to the physiological pH and under ionic conditions.

In the method according to the invention the detection is carried out by detecting a change in the optical properties of the detection fields. The optical change in the detection field can be caused, for example, by isotopes, enzymes, fluorochromes, dyes, metal colloids, beads or similar materials which serve as the label system. For the detection, one of the interaction partners, for example, the receptor or the analyte, is labeled directly or indirectly. It is preferred to derivatize, for the detection, proteins or nucleic acids with one biotin unit, which then allows identification via streptavidin conjugates.

It is preferred that the detection method results in the precipitation of a dye or the localization of a fluorochrome at the place of the molecular interaction or in the accretion of metal clusters with strong electromagnetic interaction. Latex beads or plastic beads can also be bound. Because of their refractive behavior and their curved (spherical) surface, they effect a scattering of the incident read-out light, which can be detected as a reduction of intensity. If the beads have appropriate dimensions, effects of destructive interference can be achieved.

The interacting molecules, that is the receptor and the analyte, can be detected either directly via specific binding sites, or indirectly, by being provided with a label which contains a specific binding site. Examples of such labels are epitopes for which known monoclonal antibodies exist, or a biotin group, which can combine with streptavidin. In addition, it is also possible to use direct specific binding of an antibody to the analyte. Antibodies and streptavidin are preferably conjugated with an enzyme to allow the evaluation of the detection fields. Examples of preferred enzymes are horseradish peroxidase, alkaline phosphatase and β-

galactosidase. At the time of the addition of appropriate substrates, an enzymatic chromogenic reaction occurs, during which colored products are formed which precipitate at those places where the enzymes are bound and thus indicate the presence of the analyte. Suitable substrates of the above enzymes which allow an optical evaluation are, in the case of horseradish peroxidase, for example, diaminobenzidine (DAB), which produces a brown product which is insoluble in water and ethanol, DAB + metal, which results in a gray to black insoluble product, in the presence of cobalt or nickel, chlornaphthol, which produces a blue-black water-insoluble coloration, or aminoethyl carbazole, which produces a red water-insoluble product. Preferred substrates for alkaline phosphatase are naphthol-AS-BI-phosphate/new fuchsin, which produces a red insoluble product, bromochlorindolyl phosphate/nitrotetrazolium, which produces a black-violet precipitate, and for β-galactosidase bromochlorindolyl-b-D-galactopyranosite (BCIG), which produces an insoluble blue product. The detection of the analytes can easily be carried out here by optical detection of the colored area.

An additional possibility for the detection of the interaction sites is the technique of staining with gold colloids, a technique for which other types of metal clusters or beads can also be used. Silver particles are particularly advantageous in this context, which are capable of increasing the sensitivity of an optical detection system based on nonlinear optical effects near a metal surface of the reflecting layer. Furthermore, it is preferred to use dyes as labels, particularly dyed latex particles. Glass beads made of silicon oxide which are filled with a dye at high concentration can also be used.

In addition to the measurement of the absorption, in the case where suitable labels are chosen, for example, fluorescing substances, it is also possible to measure the fluorescence where, in this case, the detection wavelength is different from the irradiated wavelength. To the extent that commercial CD drives, particularly CD-ROM drives, are to be used for the evaluation, it may be necessary, in the case of fluorescence measurements, to make changes in the construction of the drive, in addition to the corresponding adaptation of the software. In particular, it can be necessary to incorporate a detector which is specifically tuned to the fluorescence wavelength.

The invention makes it possible to record additional information on the planar face of the substrate which carries the detection fields, which information can be read and evaluated simultaneously with the detection fields. Accordingly, it is proposed to form additional data fields along the spiral line or at least one circular line of the planar face of the substrate which carries the detection fields, which data fields contain information pertaining to the sample and/or detection field and/or evaluation. In the case of information on the sample, the information can pertain, for example, to the place and time of collection of the sample, the type of sample, or the individuals from whom the sample was collected. Information concerning the detection field can

contain biomolecular or biochemical data on the detection fields, particularly on the binder type of the individual detection fields. The information pertaining to the evaluation can be data concerning the detection principle used for the detection of the analytes, for example, enzymatic detection, detection via dyes, or detection via metal clusters or similar materials. Furthermore, they can contain data indicating which physical scanning principle a reading device is to use, for example, absorption measurement or fluorescence measurement. In addition, the information pertaining to the evaluation can give to the reading device the position and place of the detection fields on the substrate, and thus it can notify the reading device of the time when it should change from software for reading data fields to software for reading detection fields. In particular, it is conceivable that the information pertaining to evaluation already contains at least parts of an evaluation software, which is retrieved by the reading device at the time of the evaluation of the detection fields. In this manner, the user does not need to carry out laborious programming tasks to adapt the software of his/her computer workplace. The entire evaluation software can already be recorded in the sensor by the manufacturer, to the extent that appropriate software standards exist.

In principle it is conceivable to arrange the detection fields and the data fields separately along the spiral line. However, it can also be advantageous to arrange the detection fields and data fields alternately along the spiral line, for example, in such a manner that an individual detection field or a group of detection fields is associated with a data field which precedes the detection field or group of detection fields along the spiral line and which provides the evaluation system with information on this detection field or this group of detection fields.

To the extent that the detection fields are arranged along concentric circular lines, detection fields and data fields can also be arranged alternately along at least one circular line. It is also conceivable to form detection fields and data fields in each case on separate circular lines.

The data fields should advantageously be read from the same side of the substrate disc as the detection fields. This makes it possible to form recesses in the planar face of the substrate which carries the detection fields for the formation of the data fields, where the reflecting layer is applied in such a manner that it penetrates into the recess. Advantageously, the coding of the data and the arrangement of the recesses meet the specifications of a standard CD format, particularly CD-ROM format, so that the data fields can be read by conventional CD drives without software modifications.

It is even conceivable to form the data fields without using recesses. By binding optically absorbing or scattering substances to the substrate it is also possible to influence the reflection of the light beam which is directed on the detection and data fields for the reading process. Thus, it is conceivable, with an appropriate choice of metal and plastic beads, to achieve similar effects of destructive interference to those which are also achieved by recesses in the substrate surface.

Therefore, it is possible to apply, for the formation of the data fields, a substance which influences incident reading light on the planar face of the substrate which carries the detection fields.

Because the reflecting layer is applied only after the completion of all biomolecular or biochemical analysis steps, it is not possible to carry out before-after measurements of the detection fields, that is measurements before and after contacting the sample with the detection fields. However, in order to be nevertheless capable of obtaining reliable information from the measurement signals concerning the presence of analytes on detection fields and analyte identification, it can be advantageous to form, in addition, at least one reference field along the spiral line or along at least one circular line of the planar face of the substrate which carries the detection fields, where the reference field has optical properties which are used as reference in the evaluation of the detection fields. For example, these reference fields can have a known reference absorption level for the light of the scanning light beam, which level is typical for analyte-free detection fields, and which is distinguishable from the absorption level which detection fields typically have if analytes adhere to them. In addition, reference fields can also be formed which contain the analyte and/or label, and which function as a positive control in the correct use of the method. The reference fields can, moreover, be used for the calibration and for the quantification of the measurements.

In the area of the detection field, there may be a decrease in the adhesion of the reflecting layer, if the latter is immediately applied to the biomolecular or biochemical plane which lies below it. In this case, it can be advantageous, after contacting the sample with the detection fields, to first apply a coating layer made of an optically transparent material onto the detection fields, before the application of the reflecting layer. The coating layer can also be used for the fixation of the reagents in the detection fields. The material and the thickness of the coating layer must be matched in such a manner that there is no influence on the optical properties of the sensor caused by the coating layer, for example, as a result of focusing or absorption effects, or, at least, so that any influence occurs in a controllable manner. A polymer-based material has been shown to be best suited for the coating layer. Thus, for example, it can be applied as a film or by a spraying method. To the extent that the sensor also has recesses in the data fields, one must ensure that the recesses are not filled back up by the material of the coating layer, to avoid a loss of data information represented by the recesses. It is conceivable to use spraying methods for the local application of the coating layer. It is also conceivable to carry out a fictitious subdivision of the surface of the substrate disc into segments, for example, sectors, of which a portion is exclusively reserved for detection fields and another portion exclusively for data fields. The segments with data fields can then easily be kept free of the coating layer.

The substrate with the binders immobilized on it can be packaged as a unit which can be handled and sent to the user. It is conceivable that the manufacturer could compile a customer-specific set of binders and apply it to the substrate. It is also conceivable to apply a set of binders to a substrate in a manner which is not specific to a customer, but specific to an application, for example, for certain gene analyses, and to offer this substrate as a prepared support. It is recommended to dry this support before packaging and shipping it. The sample to be examined is then applied by the user, that is by the purchaser of the support. The same can also apply to reagents which contain labels. The application of the reflecting layer and optionally the coating layer after completion of the biomolecular or biochemical detection method, which can also comprise washing steps, can be carried out by the user, provided he/she has appropriate equipment for this purpose. It is also conceivable that the user sends back the support with adhering analyte to the manufacture, or another laboratory, where these layers are applied.

It is also possible to apply a planar protective layer to the reflecting layer, for which protective layer an acrylate-based material is suitable because of its notch and scratch resistance. The support which has been protected in this manner can be stored for long periods and it can be read again at any time.

Furthermore, the invention relates to a support according to Claim 18, which is intended for use in the above-explained method.

According to an additional aspect a method according to Claim 23 is provided according to the invention.

The circle- or spiral-shaped distribution of the detection fields on the substrate allows the use of a commercial magnetic disc reading device, so-called hard disc drives. Optionally, an adaptation of the software of the drive controller is required. The magnetic reading of the detection fields even allows the formation of detection fields on both planar faces of the substrate, resulting in the possibility of further increasing the packing density of the sensors with detection fields. With regard to the mutual separations, the sizes and the arrangement of the detection fields, as well as of any data and reference fields on the substrate, the comments made above for the optically readable sensor essentially apply.

In order to prevent the label from being torn off as a result of contact with the reading head of the evaluation device from the substrate, a fixation layer can be applied onto the detection fields after contacting the sample with the detection field. The fixation layer should advantageously be flatly applied on each planar face of the substrate which carries detection fields. Polymer-based materials have been shown to be suitable for the fixation layer.

Prior to the formation of the detection field or after contacting the sample with the detection fields, a magnetic and/or magnetizable particle containing magnetic layer is flatly applied over each planar face of the substrate which carries detection fields. The magnetic layer

makes it possible to record additional data in the sensor, which can be read together with the detection fields. The magnetic properties of the magnetic layer will be chosen in such a manner that the local oscillations in the magnetic field strength or the magnetic flux density caused by the labels do not become "blurred" as a result of the magnetic layer, and thus become undetectable. Although, in principle, it is conceivable that the magnetic layer is applied, below the biomolecular or biochemical layer of the sensor, directly to the substrates, it is nevertheless advantageous to apply it only after the completion of the biomolecular or biochemical process steps, because it can simultaneously serve as fixation layer.

A test kit for use with the method according to the above-described first and second aspect comprises a support which has been prepared with immobilized binders, as well as reagents for use with the detection method, in particular optical and/or magnetically detectable detection reagents, as well as, optionally, washing and/or buffer solutions. The support is preferably in the dried form.

Finally, the invention relates to the use of a support of the above-described type in an immunoassay and/or nucleic acid hybridization assay and/or lectin-sugar assay and/or protein-nucleic acid assay.

The invention is further explained below with reference to the drawings in the appendix. In the drawings:

Figure 1 represents a schematic cross section of a biomolecular or biochemical sensor according to the invention ("biosensor") with detection and data fields, and

Figure 2 schematically represents the distribution of the detection and data fields on the biosensor.

In Figure 1, which does not exactly reproduce the real size ratios, the biosensor in general bears the reference numeral 1. It is in the shape of a circular disc. Advantageously, its size corresponds to that of a conventional compact disc, whose diameter is usually approximately 12 cm. The biosensor 1 comprises a carrier substrate 3 which is prepared from an optically transparent material, preferably polycarbonate. On the top planar face of substrate 3 in Figure 1, detection fields 5, 7 and data fields 9 are formed. In the data fields 9, information concerning the sample and/or the detection fields and/or the evaluation is stored. As in conventional CDs, the information in the data fields 9 is represented by alternating areas 11 with increased depth and areas 13 without increased depth of the substrate 3.

In the detection fields 5, 7, analyte-specific binders or receptors are immobilized. These binders are schematically represented in Figure 1 by short lines 15. Each detection field 5, 7 carries a plurality of binders 15 of the same or different type. The detection field 7, in Figure 1, represents a detection field to which no analytes have become bound after contacting the sample with the detection fields 5, 7. In contrast, the detection field 5 represents a detection field in

which the analytes remain bound. These analytes are schematically represented in Figure 1 by a small circles 17, which simultaneously symbolize the labels, by means of which the optical detection of the presence of the analytes is possible.

The detection fields 5, 7 are embedded in a coating layer 19, which is only applied in the areas of the substrate 3 which carry the detection fields 5, 7, but not in the areas of the substrate 3 which carry the data fields 9. The coating layer 19 also consists of an optically transparent material, preferably a polymer material. It fixes the labels to the substrate 3 and at the same time it functions as an adhesive between the substrate 3 and the reflecting layer 21, which is applied in a planar manner on the substrate 3, over all detection fields 5, 7 and all data fields 9, and which extends into the recesses 11 of the data fields 9. The reflecting layer 21 preferably is made of aluminum, but it can also be made of silver, for example, and it is advantageously produced by chemical vapor phase deposition. The reflecting layer 21 serves as a reflector for the light of a laser beam 23, which is directed from the bottom side of the substrate 3 onto the biosensor 1 for reading the detection fields 5, 7 and the data fields 9. The reflected light is evaluated using routine methods. For example, the light is separated by means of a polarization filter from the irradiated light, and then evaluated to determine its intensity. The material of the substrate 3 advantageously has a refractive index such that the laser beam 23 is focused on its entry path in the substrate 3, so that the light spot which in the end falls onto the detection fields 5, 7 and the data fields 9 can be kept very small.

Toward the top side, the biosensor 1 is closed off by a coating layer 25, which is applied in a planar manner, and which protects the biosensor 1 from harmful chemical or mechanical influences. Advantageously, it is made of an acrylate material.

The detection fields 5, 7 and the data fields 9 are arranged in an alternating sequence along a spiral line on the substrate 3. Figure 2 shows a detail of two successive windings of the spiral line. The latter is represented in Figure 2 as a broken line, and it bears the reference numeral 27. Furthermore, d denotes the radial separation between the two windings, that is the pitch of the spiral. It is, for example, approximately 1.6 μm. The recesses 11, which, in the case of the coding which is routinely used for CDs, particularly CD-ROMs, can have a gradually varying circumferential length, have, for example, a width of approximately 0.5 μm in the radial direction. To prevent radial overlapping with detection and data fields of adjacent spiral windings, the detection fields 5, 7 are also formed radially so small that there is a sufficient separation from the fields of the adjacent spiral windings. In particular, the detection fields 5, 7 can also be approximately 0.5 mm wide. In the case of such dimensions, one can without any problem tolerate the "fraying" which is frequently observed in the case of the enzymatic detection of analytes adhering in the detection fields 5. In the circumferential direction, the detection fields 5, 7 can be elongated, so that they can be formed with a sufficiently large total

surface area. In the circumferential direction as well, the detection fields 5, 7 will be at a sufficient separation from each other and from the data fields 9.

The above-mentioned measures are particularly recommended if, for reading the data and detection fields, CD drives with a spot diameter of the laser beam 23 of approximately 2 μm are used.

Below, some biomolecular or biochemical application examples of the invention are explained.

Example 1

Applications

The applications can be classified depending on the type of the immobilized binding partner (A), of the analyte-specific binder, and the type of its "ligand", that is the analyte (B). It is preferred to use nucleic acids and/or proteins as interaction partners, however, other partners of specific binding pairs can also be used, such as lectins or sugars.

It is preferred to derivatize the receptors or analytes, particularly proteins and nucleic acids, with a biotin group, where the biotin group is then advantageously detected with a streptavidin enzyme conjugate, such as horseradish peroxidase. The substrate of the enzyme reaction is chosen in such a manner that an intense dark precipitate forms at the place of the molecular interaction, which quenches the detection laser. In addition, the detection can also be carried out by direct derivatization of an interaction partner with a fluorochrome in combination with an appropriate laser.

Example 1.1

The receptor (A) is an RNA aptamer, a peptide or a natural or synthetic effector molecule, and the analyte (B) is a protein.

The properties of an enzyme or a regulation factor in gene expression are frequently modulated by the association of small effect or ligand molecules. Examples are hormone receptors, which are converted after the binding to the hormone into an active conformation. The protein function can be modulated by the interaction of a substrate analog or by allosteric effectors. To identify suitable small chemicals, screening processes for ligands are carried out, with the examination of a large number of different chemicals.

According to the method of the invention it is possible to completely test chemical libraries. In this process, a plurality of different small chemicals is immobilized in a defined order on the support, where the protein in question is contacted with the support under different

stringency conditions. Bound protein is detected directly, if it is labelled with an appropriate fluorochrome, for example, a latex particle, or indirectly using an enzyme amplification cascade. In a corresponding manner, large libraries of RNA aptamers or peptides can be screened, either separately or bound in a fusion protein, to determine interactions with a protein in question.

Example 1.2

The receptor (A) is a protein, and the analyte (B) is a nucleic acid or a ligand.

Protein libraries can be screened with respect to certain binding properties, for example, the interaction with a defined DNA sequence. Replicated supports which contain defined areas of proteins, which together represent the products of a cDNA expression library, can be characterized with specific ligands or DNA binding sites. The arranged proteins can thus be unknown proteins, as in the library, and identified only by the method according to the invention. Alternately, areas of known proteins or derivatives of a defined protein, such as the product of a random mutagenesis, can be examined.

Example 1.3

The receptor (A) is DNA or RNA, and the analyte (B) is a protein.

A large number of different double-stranded DNA fragments can be applied to the detection fields. In this context, the fragments can either be small fragments for example, oligonucleotides, or larger DNA fragments to very large DNA fragments which represent an ordered library of genomic DNA fragments. The applications comprise the systematic search for possible genomic binding sites for DNA binding proteins, but also other DNA binding molecules can be detected, such as intercalators, small molecules with a preference for certain sequences, PNAs and sequences which form a triple helix. Similarly, RNA molecules, such as transcripts of a cDNA library or the derivatives of a defined RNA, such as the product of a random mutagenesis can be applied as receptor molecules in the detection fields.

Example 2

Nucleic acid examinations

The receptor (A) is single-strand DNA, and the analyte (B) is single-strand DNA or RNA.

Example 2.1

DNA mapping

It has become possible today to sequence entire genomes, and the genomes of higher eukaryotes, such as humans, mice and fleas, are mapped and covered by ordered clones (such as P1 phage clones), where the assumption is that the sequences of all genomes will be available in the foreseeable future. In this context, supports according to the invention can be prepared which contain entire genomes in ordered arrays or areas. Thus, it is possible to assign a cloned piece of DNA in a single hybridization step to its genomic location and, depending on the resolution of the array (which is a function of the mean sequence length and the number of individual DNA molecules), even to identify the gene itself. Examples of such an application are the P1 arrays of Genome Systems, which so far had been difficult to screen, because they were only available on membranes. The application of these P1 arrays onto supports according to the invention allows a simple and uncomplicated use; translocations and other genomic rearrangements, which are frequently the cause of genetic diseases, can also be mapped in a simple way, including deletions of the mitochondrial genome. In addition, inserts of transgenes can easily be mapped if the inserted DNA is detected together with a small quantity of flanking genomic DNA.

Example 2.2

Gene identification and/or gene cloning

Arrays or areas of diagnostic oligonucleotides, which represent all known genes of a given species, allow the direct identification of a gene in question. For this purpose one can improve, for example, cDNA arrays from Klontech or DNA chips from Affimetrix, on which all the yeast genes are applied, by a transfer to the supports according to the invention.

Example 2.3

Expression profile examinations

By the application of DNA representing all the genes of an organism on supports according to the invention, the expression profile can be examined. A complex mixture of RNA is applied on the support which represents the expression state of a certain cell or of a cDNA population derived therefrom. The expression state determined on the support can easily be compared with the expression state of other cells which originate from other tissues, other developmental stages or other metabolic states. Alternately, DNA samples can be examined

which represent certain chosen DNA states, such as cell cycle genes, signal transmission components, etc. Because the supports according to the invention can be stored over a long time period, expression profiles can be prepared which can be archived and used for comparative purposes.

Example 2.4

Molecular diagnosis of DNA polymorphisms

The method according to the invention can be used for the diagnosis of common or rare DNA polymorphisms, including the mapping of mutations in protooncogenes, which cause common tumors. An appropriate support contains overlapping oligonucleotides which together comprise a complete gene (for example, the wild type sequence), together with oligonucleotides which contain all possible or frequently occurring mutations of this sequence. The corresponding DNA fragment is isolated from patients by PCR and hybridized to the relevant gene support under conditions in which the hybridization only occurs if there is a perfect match between the sequences. A comparison of the hybridization of the experimental DNA to wild type or mutant oligonucleotides makes it possible to determine with precision what type of mutation occurs in a sequence.

Example 3

In addition to the applications described above, in which a simple binding process was demonstrated which produces signals which can optionally be amplified by an enzyme cascade to increase the sensitivity of the detection, the method according to the invention can also be used in applications which require more extensive treatments of the support, for example, PCR amplification, which is carried out analogously to in situ PCR amplifications. In this manner it is also possible to detect infectious agents.

Fig. 1

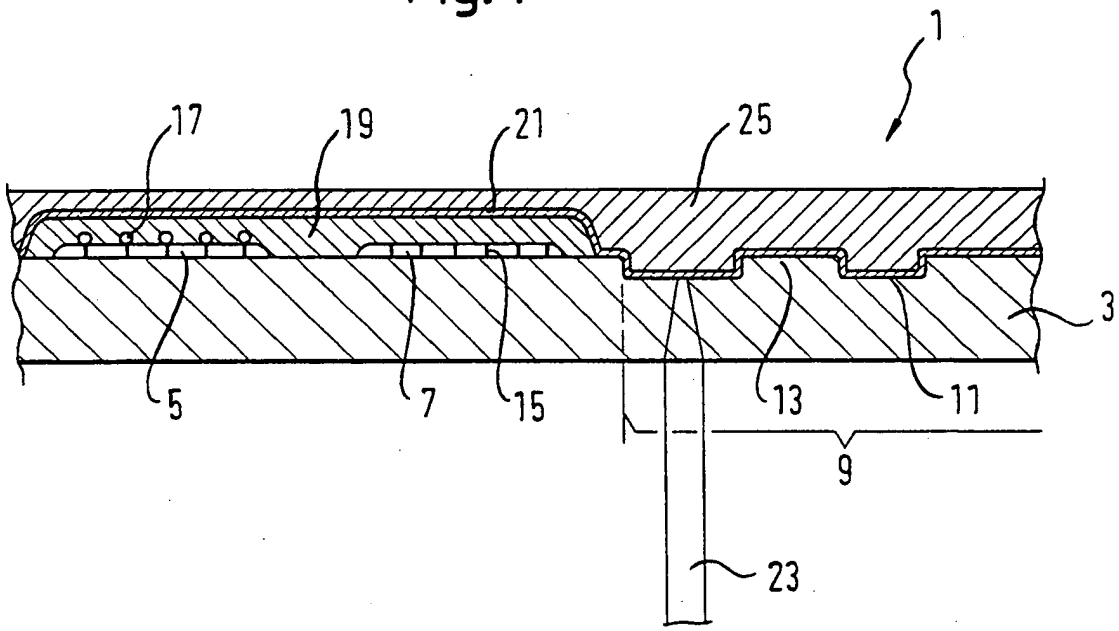
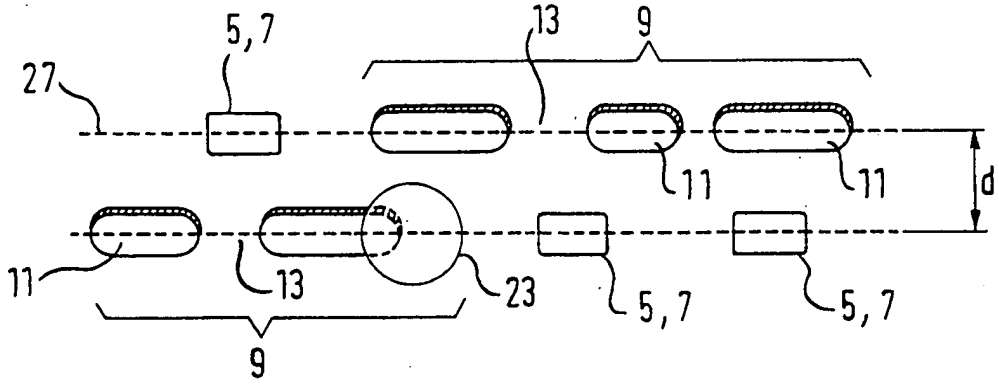
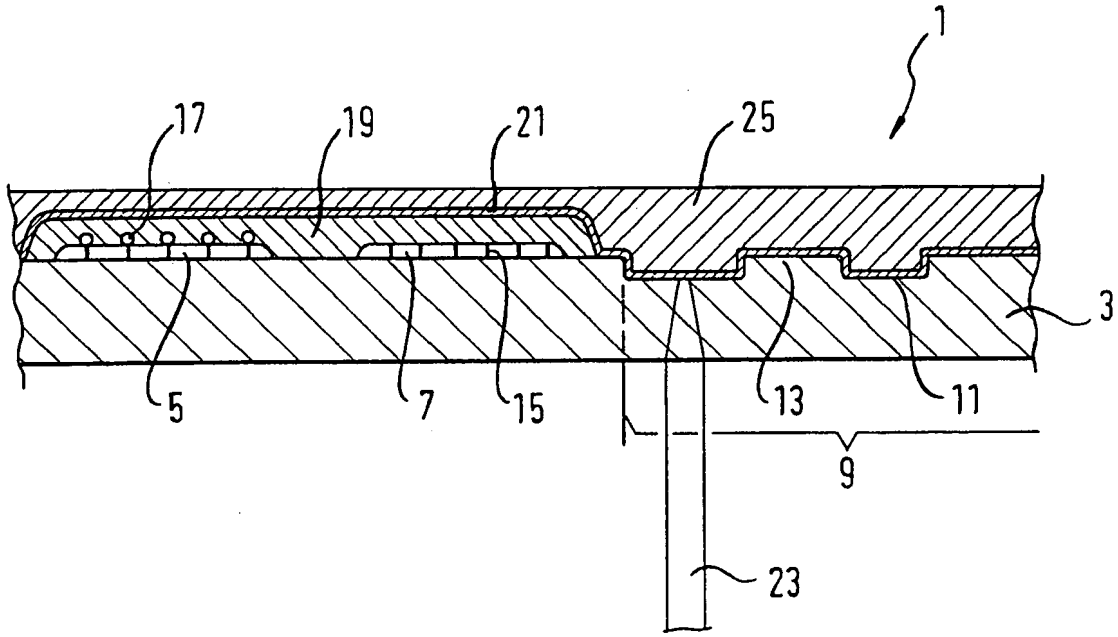



Fig. 2

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